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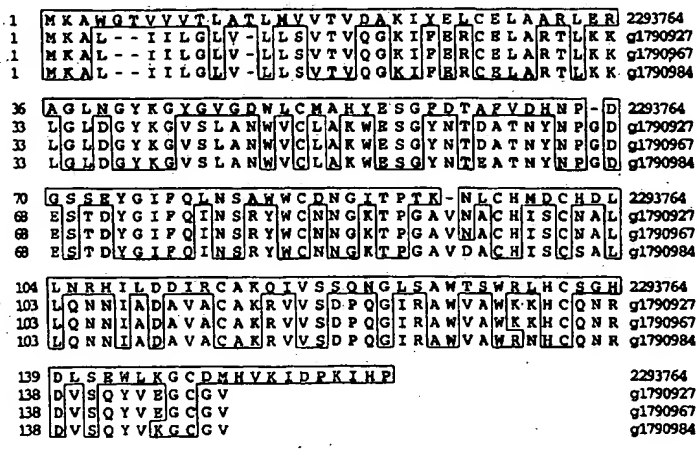

(57) Abstract

The invention provides human hydrolase proteins (HYDRL) and polynucleotides which identify and encode HYDRL. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HYDRL.

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HUMAN HYDROLASE PROTEINS

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of hydrolase proteins and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative, autoimmune/inflammatory, neurological, renal, adrenal, and genetic disorders.

BACKGROUND OF THE INVENTION

Hydrolysis is the breaking of a covalent bond in a substrate by introduction of a water molecule. The reaction involves a nucleophilic attack by the water molecule's oxygen atom on a target bond in the substrate. The water molecule is split across the target bond, breaking the bond and generating two product molecules. Hydrolases participate in reactions essential to functions such as cell signaling, cell proliferation, inflammation, apoptosis, secretion and excretion. Hydrolases are involved in key steps in disease processes involving these functions. Hydrolases, or hydrolytic enzymes, may be grouped by substrate specificity into classes including aminohydrolases, phospholipases, carboxyl-esterases, phosphodiesterases, glycosidases, glyoxalases, sulfatases, phosphohydrolases, serine hydrolases, and lysozymes.

NG,NG-dimethylarginine dimethylaminohydrolase (DDAH) is an enzyme that hydrolyzes the endogenous nitric oxide synthase (NOS) inhibitors, NG-monomethyl-arginine and NG,NG-dimethyl-L-arginine, to L-citrulline. Inhibiting DDAH can cause increased intracellular concentration of NOS inhibitors to levels sufficient to inhibit NOS. Therefore, DDAH inhibition may provide a method of NOS inhibition, and changes in the activity of DDAH could play a role in pathophysiological alterations in nitric oxide generation (MacAllister, R.J. et al. (1996) Br. J. Pharmacol. 119:1533-1540). DDAH was found in neurons displaying cytoskeletal abnormalities and oxidative stress in Alzheimer's disease. In age-matched control cases, DDAH was not found in neurons. This suggests that oxidative stress- and nitric oxide-mediated events play a role in the pathogenesis of Alzheimer's disease (Smith, M.A. et al. (1998) Free Rad. Biol. Med. 25:898-902).

Phosphodiesterases catalyze the hydrolysis of one of the two ester bonds in a phosphodiester compound. Phosphodiesterases are, therefore, crucial to a variety of cellular processes. Phosphodiesterases include DNA and RNA endo- and exo-nucleases, which are essential to cell growth and replication as well as protein synthesis.

Pancreatic lipase and colipase form a complex that plays a key role in dietary fat digestion by converting insoluble long chain triacylgycerols into more polar molecules able to cross the brush border of intestinal cells. Colipase binds to the C-terminal domain of lipase. In solution, this

interaction involves the formation of an ion pair between a glutamic acid residue of colipase and a lysine residue of lipase. These residues are strictly conserved among species (Ayvazian, L. et al. (1998) J. Biol. Chem. 273:33604-33609). Colipase appears to overcome the inhibitory effects of bile salts on pancreatic lipase (OMIM 246600 on April 28, 1999).

Carboxylesterases are proteins that hydrolyze carboxylic esters and are classified into three categories- A, B, and C. Most type-B carboxylesterases are evolutionarily related and are considered to comprise a family of proteins. The type-B carboxylesterase family of proteins includes vertebrate acetylcholinesterase, mammalian liver microsomal carboxylesterase, mammalian bile-salt-activated lipase, and duck fatty acyl-CoA hydrolase. Some members of this protein family are not catalytically active but contain a domain related evolutionarily to other type-B carboxylesterases, such as thyroglobulin and the Drosophila protein neuractin. The active site of carboxylesterases involves three residues: a serine, a glutamate or aspartate, and a histidine. The sequence surrounding this catalytic site is well conserved and can be used as a signature pattern (PROSITE: PDOC00112 at www.expasy.ch/cgi-bin/get-prodoc-entry on May 11, 1999).

Acyl-CoA thioesterase is another member of the carboxylesterase family (Alexson, S.E. et al. (1993) Eur. J. Biochem. 214:719-727). Evidence suggests that acyl-CoA thioesterase has a regulatory role in steroidogenic tissues (Finkielstein, C. et al. (1998) Eur. J. Biochem. 256:60-66).

A phospholipase A_2 inhibitor has been identified that has 33% sequence homology with human leucine-rich α_2-glycoprotein (Okumura, K. et al. (1998) J. Biol. Chem. 273:19469-19475). Leucine-rich repeat (LRR) consensus sequences have also been found in the primary structure of many proteins, including proteins that participate in biologically important processes, such as receptors for hormones, enzymes, enzyme inhibitors, proteins for cell adhesion, and ribosome-binding proteins. All proteins containing LRR domains are thought to be involved in protein-protein interactions.

The glyoxylase system consists of glyoxalase I, which catalyzes the formation of S-D-lactoylglutathione from methyglyoxal, a side product of triose-phosphate energy metabolism, and glyoxylase II, which hydrolyzes S-D-lactoylglutathione to D-lactic acid and reduced glutathione. Methyglyoxal levels are elevated during hyperglycemia, likely due to increased triose-phosphate energy metabolism. Elevated levels of glyoxylase II activity have been found in human non-insulin-dependent diabetes mellitus and in a rat model of this disease. The glyoxylase system has been implicated in the detoxification of bacterial toxins, and in the control of cell proliferation and microtubule assembly. Elevated levels of S-D-lactoylglutathione, the substrate of glyoxylase II, induced growth arrest and toxicity in HL60 cells. Thus, the glyoxylase system, and glyoxylase II in particular, may be associated with cell proliferation and autoimmune disorders such as diabetes.

The alpha/beta hydrolase protein fold is common to several hydrolases of diverse phylogenetic origin and catalytic function. Enzymes with the alpha/beta hydrolase fold have a common core structure consisting of eight beta-sheets connected by alpha-helices. The most conserved structural feature of this fold is the loops of the nucleophile-histidine-acid catalytic triad. The histidine in the catalytic triad is completely conserved, while the nucleophile and acid loops accommodate more than one type of amino acid (Ollis, D.L. et al. (1992) Protein Eng. 5:197-211).

Sulfatases are members of a highly conserved gene family that share extensive sequence homology and a high degree of structural similarity. Sulfatases catalyze the cleavage of sulfate esters. To perform this function, sulfatases undergo a unique post-translational modification in the endoplasmic reticulum that involves the oxidation of a conserved cysteine residue. A human disorder called multiple sulfatase deficiency is due to a defect in this post-translational modification step, leading to inactive sulfatases (Recksiek, M. et al. (1998) J. Biol. Chem. 273:6096-6103).

Phosphohydrolases are enzymes that hydrolyze phosphate esters. Some phosphohydrolases contain a mutT domain signature sequence. MutT is a protein involved in the GO system responsible for removing an oxidatively damaged form of guanine from DNA. A region of about 40 amino acid residues, found in the N-terminus of mutT, is also found in other proteins, including some phosphohydrolases (PROSITE: PDOC00695 at www.expasy.ch/cgi-bin/get-prodoc-entry on April 27, 1999).

Glycosidases catalyze the cleavage of hemiacetyl bonds of glycosides, which are compounds that contain one or more sugar. Mammalian beta-galactosidase removes the terminal galactose from gangliosides, glycoproteins, and glycosaminoglycans. Beta-galactosidases belong to family 35 in the classification of glycosyl hydrolases. Deficiency of this enzyme is associated with the genetic disease GM1-gangliosidosis, also known as Morquio disease type B (PROSITE: PDOC00910 at www.expasy.ch/cgi-bin/get-prodoc-entry on May 12, 1999).

Serine hydrolases are a functional class of hydrolytic enzymes that contain a serine residue in their active site. This class of enzymes contains proteinases, esterases, and lipases which hydrolyze a variety of substrates and, therefore, have different biological roles. Proteins in this superfamily can be further grouped into subfamilies based on substrate specificity or amino acid similarities (Puente, X.S. and C. Lopez-Ont (1995) J. Biol. Chem. 270:12926-12932). One member of the serine hydrolase superfamily is kraken, a Drosophila gene isolated from a Drosophila embryo cDNA library. Kraken belongs to a subfamily whose members catalyze cleavage of substrates with a carbonyl-containing group (Chan, E. et al. (1998) Gene 222:195-201).

The lysozyme c superfamily consists of conventional lysozymes c, calcium-binding lysozymes c, and α-lactalbumin (Prager, E.M. and P. Jolles (1996) EXS 75:9-31). The proteins in this

superfamily have 35-40% sequence homology and share a common three-dimensional fold, but can have different functions. Lysozymes c are ubiquitous in a variety of tissues and secretions and can lyse the cell walls of certain bacteria (McKenzie, H.A. (1996) EXS 75:365-409). Alpha-lactalbumin is a metallo-protein that binds calcium and participates in the synthesis of lactose (Iyer, L.K. and P.K. Qasba (1999) Protein Eng. 12:129-139). Alpha-lactalbumin occurs in mammalian milk and colostrum (McKenzie, supra).

Lysozymes catalyze the hydrolysis of certain mucopolysaccharides of bacterial cell walls, specifically, the beta (1-4) glycosidic linkages between N-acetylmuramic acid and N-acetylglucosamine, and cause bacterial lysis. Lysozymes occur in diverse organisms including viruses, birds, and mammals. In humans, lysozymes are found in spleen, lung, kidney, white blood cells, plasma, saliva, milk, tears, and cartilage (Online Mendelian Inheritance in Man (OMIM) #153450 Lysozyme; Weaver, L.H. et al. (1985) J. Mol. Biol. 184:739-741). Lysozyme c functions in ruminants as a digestive enzyme, releasing proteins from ingested bacterial cells, and may perform the same function in human newborns (Braun, O.H. et al. (1995) Klin. Pediatr. 207:4-7).

The two known forms of lysozymes, chicken-type and goose-type, were originally isolated from chicken and goose egg white, respectively. Chicken-type and goose-type lysozymes have similar three-dimensional structures, but different amino acid sequences (Nakano, T. and T. Graf (1991) Biochim. Biophys. Acta 1090:273-276). In chickens, both forms of lysozyme are found in neutrophil granulocytes (heterophils), but only chicken-type lysozyme is found in egg white. Generally, chicken-type lysozyme mRNA is found in both adherent monocytes and macrophages and nonadherent promyelocytes and granulocytes as well as in cells of the bone marrow, spleen, bursa, and oviduct. Goose-type lysozyme mRNA is found in non-adherent cells of the bone marrow and lung. Several isozymes have been found in rabbits, including leukocytic, gastrointestinal, and possibly lymphoepithelial forms (OMIM #153450, supra; Nakano and Graf, supra; and GenBank GI 1310929). A human lysozyme gene encoding a protein similar to chicken-type lysozyme has been cloned (Yoshimura, K. et al. (1988) Biochem. Biophys. Res. Commun. 150:794-801). A consensus motif featuring regularly spaced cysteine residues has been derived from the lysozyme C enzymes of various species (Prosite PS00128, *http://expasy.hcuge.ch* Swiss Institute of Bioinformatics). Lysozyme C shares about 40% amino acid sequence identity with α-lactalbumin.

Lysozymes have several disease associations. Lysozymuria is observed in diabetic nephropathy (Shima, M. et al.(1986) Clin. Chem. 32:1818-1822), endemic nephropathy (Bruckner, I. et al. (1978) Med. Interne. 16:117-125), urinary tract infections (Heidegger, H. (1990) Minerva Ginecol. 42:243-250), and acute monocytic leukemia (Shaw, M.T. (1978) Am. J. Hematol. 4:97-103). Nakano (supra) suggested a role for lysozyme in host defense systems. Older rabbits with an

inherited lysozyme deficiency show increased susceptibility to infections, such as subcutaneous abscesses (OMIM #153450, supra). Human lysozyme gene mutations cause hereditary systemic amyloidosis, a rare autosomal dominant disease in which amyloid deposits form in the viscera, including the kidney, adrenal glands, spleen, and liver. This disease is usually fatal by the fifth decade. The amyloid deposits contain variant forms of lysozyme. Renal amyloidosis is the most common and potentially the most serious form of organ involvement (Pepys, M.B. et al. (1993) Nature 362:553-557; OMIM #105200 Familial Visceral Amyloidosis; Cotran, R.S. et al. (1994) Robbins Pathologic Basis of Disease, W.B. Saunders Company, Philadelphia PA, pp. 231-238). Increased levels of lysozyme and lactate have been observed in the cerebrospinal fluid of patients with bacterial meningitis (Ponka, A. et al. (1983) Infection 11:129-131). Acute monocytic leukemia is characterized by massive lysozymuria (Den Tandt, W.R. (1988) Int. J. Biochem. 20:713-719).

The discovery of new hydrolase proteins and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of cell proliferative, autoimmune/inflammatory, neurological, renal, adrenal, and genetic disorders.

SUMMARY OF THE INVENTION

The invention features substantially purified polypeptides, hydrolase proteins, referred to collectively as "HYDRL" and individually as "HYDRL-1," "HYDRL-2," "HYDRL-3," "HYDRL-4," "HYDRL-5," "HYDRL-6," "HYDRL-7," "HYDRL-8," "HYDRL-9," "HYDRL-10," "HYDRL-11," "HYDRL-12," "HYDRL-13," "HYDRL-14," "HYDRL-15," and "HYDRL-16." In one aspect, the invention provides a substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-16 and fragments thereof. The invention also includes a polypeptide comprising an amino acid sequence that differs by one or more conservative amino acid substitutions from an amino acid sequence selected from the group consisting of SEQ ID NO:1-16.

The invention further provides a substantially purified variant having at least 90% amino acid identity to at least one of the amino acid sequences selected from the group consisting of SEQ ID NO:1-16 and fragments thereof. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-16 and fragments thereof. The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-16 and fragments thereof.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes

under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-16 and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-16 and fragments thereof.

The invention also provides a method for detecting a polynucleotide in a sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide sequence to at least one of the polynucleotides of the sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide in the sample. In one aspect, the method further comprises amplifying the polynucleotide prior to hybridization.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:17-32 and fragments thereof. The invention further provides an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide sequence selected from the group consisting of SEQ ID NO:17-32 and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:17-32 and fragments thereof.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-16. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing a polynucleotide of the invention under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-16 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide selected from the group consisting of SEQ ID NO:1-16 and fragments thereof. The invention also provides a purified agonist and a purified antagonist to the polypeptide.

The invention also provides a method for treating or preventing a disorder associated with

decreased expression or activity of HYDRL, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-16 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention also provides a method for treating or preventing a disorder associated with increased expression or activity of HYDRL, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-16 and fragments thereof.

BRIEF DESCRIPTION OF THE FIGURE AND TABLES

Figure 1 shows the amino acid sequence alignment between HYDRL-1 (Incyte Clone ID 2293764; SEQ ID NO:1), Colobus guereza lysozyme-c precursor (GI 1790927; SEQ ID NO:33), Colobus angolensis lysozyme-c precursor (GI 1790967; SEQ ID NO:34), and Nasalis larvatis lysozyme-c precursor (GI 1790984; SEQ ID NO:35), produced using the multisequence alignment program of LASERGENE software (DNASTAR, Madison WI).

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding HYDRL.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of HYDRL.

Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding HYDRL were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze HYDRL, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will

be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"HYDRL" refers to the amino acid sequences of substantially purified HYDRL obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of HYDRL. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HYDRL either by directly interacting with HYDRL or by acting on components of the biological pathway in which HYDRL participates.

An "allelic variant" is an alternative form of the gene encoding HYDRL. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding HYDRL include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as HYDRL or a polypeptide with at least one functional characteristic of HYDRL. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HYDRL, and improper or unexpected hybridization to allelic

variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HYDRL. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HYDRL. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of HYDRL is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of HYDRL. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HYDRL either by directly interacting with HYDRL or by acting on components of the biological pathway in which HYDRL participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, $F(ab')_2$, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind HYDRL polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HYDRL, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" and "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3'-T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acid strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding HYDRL or fragments of HYDRL may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been resequenced to

resolve uncalled bases, extended using the XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of one or more Incyte Clones and, in some cases, one or more public domain ESTs, using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

Original Residue	Conservative Substitution
Ala	Gly, Ser
Arg	His, Lys
Asn	Asp, Gln, His
Asp	Asn, Glu
Cys	Ala, Ser
Gln	Asn, Glu, His
Glu	Asp, Gln, His
Gly	Ala
His	Asn, Arg, Gln, Glu
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	His, Met, Leu, Trp, Tyr
Ser	Cys, Thr
Thr	Ser, Val
Trp	Phe, Tyr
Tyr	His, Phe, Trp
Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative

polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "fragment" is a unique portion of HYDRL or the polynucleotide encoding HYDRL which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:17-32 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:17-32, for example, as distinct from any other sequence in the same genome. A fragment of SEQ ID NO:17-32 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:17-32 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:17-32 and the region of SEQ ID NO:17-32 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-16 is encoded by a fragment of SEQ ID NO:17-32. A fragment of SEQ ID NO:1-16 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-16. For example, a fragment of SEQ ID NO:1-16 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-16. The precise length of a fragment of SEQ ID NO:1-16 and the region of SEQ ID NO:1-16 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

The term "similarity" refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the

completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequence pairs.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at http://www.ncbi.nlm.nih.gov/BLAST/. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at http://www.ncbi.nlm.nih.gov/gorf/bl2.html. The

"BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

Penalty for mismatch: -2

Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 11

Filter: on

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the hydrophobicity and acidity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default

residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Open Gap: 11 and Extension Gap: 1 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 3

Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term "humanized antibody" refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of identity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive

annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Generally, such wash temperatures are selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term "microarray" refers to an arrangement of distinct polynucleotides on a substrate.

The terms "element" and "array element" in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate" refers to a change in the activity of HYDRL. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of HYDRL.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Probe" refers to nucleic acid sequences encoding HYDRL, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for

example Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel et al.,1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a

recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be use to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding HYDRL, or fragments thereof, or HYDRL itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

"Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an

autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

THE INVENTION

The invention is based on the discovery of new human hydrolase proteins (HYDRL), the polynucleotides encoding HYDRL, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative, autoimmune/inflammatory, neurological, renal, adrenal, and genetic disorders.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding HYDRL. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each HYDRL were identified, and column 4 shows the cDNA

libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. The Incyte clones in column 5 were used to assemble the consensus nucleotide sequence of each HYDRL and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The methods of column 7 were used to characterize each polypeptide through sequence homology and protein motifs.

As shown in Figure 1, HYDRL-1 has chemical and structural similarity with Colobus guereza lysozyme-c precursor (GI 1790927; SEQ ID NO:33), Colobus angolensis lysozyme-c precursor (GI 1790967; SEQ ID NO:34) and Nasalis larvatis lysozyme-c precursor (GI 1790984; SEQ ID NO:35). In particular, HYDRL-1 and Colobus guereza lysozyme-c precursor share 40% identity, HYDRL-1 and Colobus angolensis lysozyme-c precursor share 40% identity, and HYDRL-1 and Nasalis larvatis lysozyme-c precursor share 41% identity.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding HYDRL. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:17-32 and to distinguish between SEQ ID NO:17-32 and related polynucleotide sequences. The polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express HYDRL as a fraction of total tissues expressing HYDRL. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing HYDRL as a fraction of total tissues expressing HYDRL. Column 5 lists the vectors used to subclone each cDNA library.

Northern analysis of SEQ ID NO:17 shows the expression of this sequence in tissue associated with cancer.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding HYDRL were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

SEQ ID NO:18 maps to chromosome 6 within the interval from 42.30 to 45.40 centiMorgans, to chromosome 9 within the interval from 130.40 to 166.50 centiMorgans, and to chromosome 16 within the interval from 88.10 to 92.60 centiMorgans.

SEQ ID NO:25 maps to chromosome 1 within the interval from 22.90 to 39.90 centiMorgans and to chromosome 3 within the interval from 30.90 to 43.00 centiMorgans. The interval on chromosome 3 from 30.90 to 43.00 centiMorgans also contains an EST associated with von Hippel-Lindau syndrome.

SEQ ID NO:28 maps to chromosome 10 within the interval from 137.60 to 139.20 centiMorgans.

The invention also encompasses HYDRL variants. A preferred HYDRL variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the HYDRL amino acid sequence, and which contains at least one functional or structural characteristic of HYDRL.

The invention also encompasses polynucleotides which encode HYDRL. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:17-32, which encodes HYDRL.

The invention also encompasses a variant of a polynucleotide sequence encoding HYDRL. In particular, such a variant polynucleotide sequence will have at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HYDRL. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:17-32 which has at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:17-32. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of HYDRL.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HYDRL, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HYDRL, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HYDRL and its variants are generally capable

of hybridizing to the nucleotide sequence of the naturally occurring HYDRL under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HYDRL or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HYDRL and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HYDRL and HYDRL derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HYDRL or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:17-32 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Perkin-Elmer). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Perkin-Elmer), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding HYDRL may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be

present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HYDRL may be cloned in recombinant DNA molecules that direct expression of HYDRL, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HYDRL.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HYDRL-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding HYDRL may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223; and Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.) Alternatively, HYDRL itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of HYDRL, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

In order to express a biologically active HYDRL, the nucleotide sequences encoding HYDRL or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding HYDRL. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of

sequences encoding HYDRL. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding HYDRL and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HYDRL and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding HYDRL. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HYDRL. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HYDRL can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding HYDRL into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HYDRL are needed, e.g. for the production of antibodies, vectors which direct high level expression of HYDRL may be used. For example, vectors

containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of HYDRL. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; and Scorer, C.A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of HYDRL. Transcription of sequences encoding HYDRL may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HYDRL may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HYDRL in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of HYDRL in cell lines is preferred. For example, sequences encoding HYDRL can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the

introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* and *apr* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), ß glucuronidase and its substrate ß-glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HYDRL is inserted within a marker gene sequence, transformed cells containing sequences encoding HYDRL can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HYDRL under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding HYDRL and that express HYDRL may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of HYDRL using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques

include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HYDRL is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HYDRL include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HYDRL, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HYDRL may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HYDRL may be designed to contain signal sequences which direct secretion of HYDRL through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct

modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HYDRL may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HYDRL protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HYDRL activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HYDRL encoding sequence and the heterologous protein sequence, so that HYDRL may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HYDRL may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, ^{35}S-methionine.

Fragments of HYDRL may be produced not only by recombinant means, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra, pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A peptide synthesizer (Perkin-Elmer). Various fragments of HYDRL may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of HYDRL and hydrolase proteins. In addition, the expression of HYDRL is closely associated with proliferating tissues, inflamed tissues, neurological tissues, and cancer. In some cases, sequences encoding HYDRL map to chromosomal regions associated with inherited diseases.

Therefore, HYDRL appears to play a role in cell proliferative, autoimmune/inflammatory, neurological, renal, adrenal, and genetic disorders. In the treatment of disorders associated with increased HYDRL expression or activity, it is desirable to decrease the expression or activity of HYDRL. In the treatment of disorders associated with decreased HYDRL expression or activity, it is desirable to increase the expression or activity of HYDRL.

Therefore, in one embodiment, HYDRL or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HYDRL. Examples of such disorders include, but are not limited to, a cell proliferative disorder, such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and a cancer including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an autoimmune/inflammatory disorder, such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphonpeia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; trauma, and hematopoietic cancer including lymphoma, leukemia, and myeloma; a neurological disorder, such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders,

progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorder of the central nervous system, cerebral palsy, a neuroskeletal disorder, an autonomic nervous system disorder, a cranial nerve disorder, a spinal cord disease, muscular dystrophy and other neuromuscular disorder, a peripheral nervous system disorder, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathy; myasthenia gravis, periodic paralysis; a mental disorder including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; a renal disorder such as renal amyloidosis, hypertension; primary aldosteronism; Addison's disease; renal failure; glomerulonephritis; chronic glomerulonephritis; tubulointerstitial nephritis; cystic disorders of the kidney and dysplastic malformations such as polycystic disease, renal dysplasias, and cortical or medullary cysts; inherited polycystic renal diseases (PRD), such as recessive and autosomal dominant PRD; medullary cystic disease; medullary sponge kidney and tubular dysplasia; Alport's syndrome; non-renal cancers which affect renal physiology, such as bronchogenic tumors of the lungs or tumors of the basal region of the brain; multiple myeloma; adenocarcinomas of the kidney; and metastatic renal carcinoma; an adrenal disorder such as angina, anaphylactic shock, arrhythmias, asthma, cardiovascular shock, Cushing's syndrome, hypertension, hypoglycemia, myocardial infarction, migraine, and pheochromocytoma; a genetic disorder, such as GM1-gangliosidosis, Niemann-Pick disease, adrenoleukodystrophy, Alport's syndrome, choroideremia, Duchenne and Becker muscular dystrophy, Down's syndrome, cystic fibrosis, chronic granulomatous disease, Gaucher's disease, Huntington's chorea, Marfan's syndrome, muscular dystrophy, myotonic dystrophy, pycnodysostosis, Refsum's syndrome, retinoblastoma, sickle cell anemia, thalassemia, Werner syndrome, von Willebrand's disease, von Hippel-Lindau syndrome, Wilms' tumor, Zellweger syndrome, peroxisomal acyl-CoA oxidase deficiency, peroxisomal thiolase deficiency, peroxisomal bifunctional protein deficiency, mitochondrial carnitine palmitoyl transferase and carnitine deficiency, mitochondrial very-long-chain acyl-CoA dehydrogenase deficiency, mitochondrial medium-chain acyl-CoA dehydrogenase deficiency, mitochondrial short-chain acyl-CoA dehydrogenase deficiency, mitochondrial electron transport flavoprotein and electron transport flavoprotein:ubiquinone oxidoreductase deficiency, mitochondrial

trifunctional protein deficiency, and mitochondrial short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency.

In another embodiment, a vector capable of expressing HYDRL or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HYDRL including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified HYDRL in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HYDRL including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of HYDRL may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HYDRL including, but not limited to, those listed above.

In a further embodiment, an antagonist of HYDRL may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HYDRL. Examples of such disorders include, but are not limited to, those cell proliferative, autoimmune/inflammatory, neurological, renal, adrenal, and genetic disorders described above. In one aspect, an antibody which specifically binds HYDRL may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HYDRL.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HYDRL may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HYDRL including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HYDRL may be produced using methods which are generally known in the art. In particular, purified HYDRL may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HYDRL. Antibodies to HYDRL may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit

dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HYDRL or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HYDRL have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HYDRL amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to HYDRL may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HYDRL-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for HYDRL may also be generated. For example, such fragments include, but are not limited to, $F(ab')_2$ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HYDRL and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HYDRL epitopes is generally used, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for HYDRL. Affinity is expressed as an association constant, K_a, which is defined as the molar concentration of HYDRL-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple HYDRL epitopes, represents the average affinity, or avidity, of the antibodies for HYDRL. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular HYDRL epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the HYDRL-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of HYDRL, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington, DC; Liddell, J.E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of HYDRL-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g.,

Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding HYDRL, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding HYDRL may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HYDRL. Thus, complementary molecules or fragments may be used to modulate HYDRL activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding HYDRL.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding HYDRL. (See, e.g., Sambrook, supra; Ausubel, 1995, supra.)

Genes encoding HYDRL can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding HYDRL. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding HYDRL. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may be employed. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of

RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HYDRL.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding HYDRL. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and

monkeys.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of HYDRL, antibodies to HYDRL, and mimetics, agonists, antagonists, or inhibitors of HYDRL. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures.

Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acids. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of HYDRL, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The

determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example HYDRL or fragments thereof, antibodies of HYDRL, and agonists, antagonists or inhibitors of HYDRL, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the LD_{50}/ED_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind HYDRL may be used for the diagnosis of disorders characterized by expression of HYDRL, or in assays to monitor patients being treated with HYDRL or agonists, antagonists, or inhibitors of HYDRL. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for HYDRL include methods which utilize the antibody and a label to detect HYDRL in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring HYDRL, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of HYDRL expression. Normal or standard values for HYDRL expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to HYDRL under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of HYDRL expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HYDRL may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of HYDRL may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HYDRL, and to monitor regulation of HYDRL levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HYDRL or closely related molecules may be used to identify nucleic acid sequences which encode HYDRL. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding HYDRL, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the HYDRL encoding sequences. The hybridization probes of the subject

invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:17-32 or from genomic sequences including promoters, enhancers, and introns of the HYDRL gene.

Means for producing specific hybridization probes for DNAs encoding HYDRL include the cloning of polynucleotide sequences encoding HYDRL or HYDRL derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HYDRL may be used for the diagnosis of disorders associated with expression of HYDRL. Examples of such disorders include, but are not limited to, a cell proliferative disorder, such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and a cancer including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an autoimmune/inflammatory disorder, such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphonpeia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections;

trauma, and hematopoietic cancer including lymphoma, leukemia, and myeloma; a neurological disorder, such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorder of the central nervous system, cerebral palsy, a neuroskeletal disorder, an autonomic nervous system disorder, a cranial nerve disorder, a spinal cord disease, muscular dystrophy and other neuromuscular disorder, a peripheral nervous system disorder, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathy; myasthenia gravis, periodic paralysis; a mental disorder including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; a renal disorder such as renal amyloidosis, hypertension; primary aldosteronism; Addison's disease; renal failure; glomerulonephritis; chronic glomerulonephritis; tubulointerstitial nephritis; cystic disorders of the kidney and dysplastic malformations such as polycystic disease, renal dysplasias, and cortical or medullary cysts; inherited polycystic renal diseases (PRD), such as recessive and autosomal dominant PRD; medullary cystic disease; medullary sponge kidney and tubular dysplasia; Alport's syndrome; non-renal cancers which affect renal physiology, such as bronchogenic tumors of the lungs or tumors of the basal region of the brain; multiple myeloma; adenocarcinomas of the kidney; and metastatic renal carcinoma; an adrenal disorder such as angina, anaphylactic shock, arrhythmias, asthma, cardiovascular shock, Cushing's syndrome, hypertension, hypoglycemia, myocardial infarction, migraine, and pheochromocytoma; a genetic disorder, such as GM1-gangliosidosis, Niemann-Pick disease, adrenoleukodystrophy, Alport's syndrome, choroideremia, Duchenne and Becker muscular dystrophy, Down's syndrome, cystic fibrosis, chronic granulomatous disease, Gaucher's disease, Huntington's chorea, Marfan's syndrome, muscular dystrophy, myotonic dystrophy, pycnodysostosis, Refsum's syndrome, retinoblastoma, sickle cell anemia, thalassemia, Werner syndrome, von Willebrand's disease, von Hippel-Lindau syndrome, Wilms' tumor, Zellweger syndrome, peroxisomal acyl-CoA oxidase deficiency, peroxisomal thiolase deficiency, peroxisomal bifunctional protein deficiency, mitochondrial carnitine

palmitoyl transferase and carnitine deficiency, mitochondrial very-long-chain acyl-CoA dehydrogenase deficiency, mitochondrial medium-chain acyl-CoA dehydrogenase deficiency, mitochondrial short-chain acyl-CoA dehydrogenase deficiency, mitochondrial electron transport flavoprotein and electron transport flavoprotein:ubiquinone oxidoreductase deficiency, mitochondrial trifunctional protein deficiency, and mitochondrial short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. The polynucleotide sequences encoding HYDRL may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered HYDRL expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HYDRL may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding HYDRL may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding HYDRL in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of HYDRL, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HYDRL, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or

overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HYDRL may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding HYDRL, or a fragment of a polynucleotide complementary to the polynucleotide encoding HYDRL, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

Methods which may also be used to quantify the expression of HYDRL include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding HYDRL may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial

chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding HYDRL on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, HYDRL, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HYDRL and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with HYDRL, or fragments thereof, and washed. Bound HYDRL is then detected by methods well known in the art. Purified HYDRL can also be coated directly onto plates for use in the aforementioned drug screening techniques.

Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HYDRL specifically compete with a test compound for binding HYDRL. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HYDRL.

In additional embodiments, the nucleotide sequences which encode HYDRL may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. [Attorney Docket No. PF-0634 P, filed November 12, 1998] and U.S. Ser. No. 60/135,519, are hereby expressly incorporated by reference.

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP

vector system (Stratagene) or SUPERSCRIPT plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), or pINCY (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent E. coli cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5α, DH10B, or ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Perkin-Elmer) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides

were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Perkin-Elmer) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VI.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programing, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:17-32. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\%\text{ sequence identity} \times \%\text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding HYDRL occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

V. Chromosomal Mapping of HYDRL Encoding Polynucleotides

The cDNA sequences which were used to assemble SEQ ID NO:18-32 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched

SEQ ID NO:18-32 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 5). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO:, to that map location.

The genetic map locations of SEQ ID NO:18, SEQ ID NO:25, and SEQ ID NO:28 are described in The Invention as ranges, or intervals, of human chromosomes. More than one map location is reported for SEQ ID NO:18 and SEQ ID NO:25, indicating that previously mapped sequences having similarity, but not complete identity, to SEQ ID NO:18 and SEQ ID NO:25 were assembled into their respective clusters. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Diseases associated with the public and Incyte sequences located within the indicated intervals are also reported in the Invention where applicable.

VI. Extension of HYDRL Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:17-32 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+}, $(NH_4)_2SO_4$, and β-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer

pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 µl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 µl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 μl to 10 μl aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent E. coli cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethysulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:17-32 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an

appropriate genomic library.

VII. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:17-32 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10^7 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

VIII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an

appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

IX. Complementary Polynucleotides

Sequences complementary to the HYDRL-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HYDRL. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of HYDRL. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HYDRL-encoding transcript.

X. Expression of HYDRL

Expression and purification of HYDRL is achieved using bacterial or virus-based expression systems. For expression of HYDRL in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express HYDRL upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of HYDRL in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding HYDRL by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, HYDRL is synthesized as a fusion protein with, e.g., glutathione

S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from HYDRL at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified HYDRL obtained by these methods can be used directly in the following activity assay.

XI. Demonstration of HYDRL Activity

For purposes of example, assays measuring the β-glucosidase activity and the lysozyme activity of an HYDRL molecule are described. In a β-glucosidase activity assay, varying amounts of HYDRL are incubated with 1 mM 4-nitrophenyl β-D-glycopyranoside (a substrate) in 50 mM sodium acetate buffer, pH 5.0, for various times (typically 1-5 minutes) at 37°C. The reaction is halted by heating to 100°C for 2 minutes. The absorbance is measured spectrophotometrically at 410 nm, and is proportional to the β-glucosidase activity of HYDRL in the sample. (See, e.g., Hrmova, M. et al. (1998) J. Biol. Chem. 273:11134-11143.)

Lysozyme activity of HYDRL is demonstrated by its ability to lyse Micrococcus lysodeikticus bacterial cells. (See, e.g., Enzymatic Assay of Lysozyme 1, Sigma Aldrich, St. Louis MO). A 0.015% suspension of lyophilized Micrococcus lysodeikticus cells (ATCC 4698) is prepared in 66 mM potassium phosphate buffer, pH 6.24 (Buffer A) at 25°C. A 2.5 ml aliquot of the cell suspension is pipetted into a optical cuvette and equilibrated to 25°C. The absorbance at 450 nm is monitored until constant, between 0.6 and 0.7, using a thermostatted spectrophotometer. A blank reaction is prepared in a second cuvette containing 2.5 ml Buffer A. HYDRL is dissolved in cold Buffer A. A 0.1 ml aliquot of the HYDRL solution is added to the test cuvette, and 0.1 ml Buffer A is added to the blank cuvette. The cuvettes are immediately mixed by inversion, and the decrease in absorbance at 450 nm is recorded for approximately 5 minutes. As the bacteria lyse, the turbidity of the solution, and hence the absorbance at 450 nm, decrease. The rate of the decrease in absorbance at 450 nm in the test cuvette is proportional to the lysozyme activity of HYDRL in the original sample.

XII. Functional Assays

HYDRL function is assessed by expressing the sequences encoding HYDRL at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a

mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10 μg of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2 μg of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of HYDRL on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding HYDRL and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding HYDRL and other genes of interest can be analyzed by northern analysis or microarray techniques.

XIII. Production of HYDRL Specific Antibodies

HYDRL substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) Methods Enzymol. 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the HYDRL amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for

selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-HYDRL activity by, for example, binding the peptide or HYDRL to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIV. Purification of Naturally Occurring HYDRL Using Specific Antibodies

Naturally occurring or recombinant HYDRL is substantially purified by immunoaffinity chromatography using antibodies specific for HYDRL. An immunoaffinity column is constructed by covalently coupling anti-HYDRL antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HYDRL are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HYDRL (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HYDRL binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HYDRL is collected.

XV. Identification of Molecules Which Interact with HYDRL

HYDRL, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HYDRL, washed, and any wells with labeled HYDRL complex are assayed. Data obtained using different concentrations of HYDRL are used to calculate values for the number, affinity, and association of HYDRL with the candidate molecules.

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are

obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Protein SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	17	2293764	BRAINON01	2293764H1 (BRAINON01), 2293764R6 (BRAINON01), 2293764T6 (BRAINON01)
2	18	949738	PANCNOT05	938735H1 (CERVNOT01), 938735X13 (CERVNOT01), 949738H1 (PANCNOT05), 1500746F1 (SINTBST01), 2206160F6 (SPLNFET02)
3	19	1297034	BRSTNOT07	1297034H1 (BRSTNOT07), 1713735F6 (UCMCNOT02), 1713735T6 (UCMCNOT02), 1889362F6 (BLADTUT07), 822634R1 (KERANOT02), 903616R2 (COLNNOT07), SAQA00517F1, SAQA00539F1
4	20	1553276	BLADTUT04	1553276F6 (BLADTUT04), 1553276H1 (BLADTUT04)
5	21	1702211	BLADTUT05	1702211H1 (BLADTUT05), 2768772F6 (COLANOT02), SCCA04943V1, SCCA02992V1, SCCA02905V1, SCCA03592V1, SCCA05380V1
6	22	1859618	PROSNOT18	168238H1 (LIVRNOT01), 294798R6 (LIVRNOT04), 1859618F6 (PROSNOT18), 1859618H1 (PROSNOT18), SARB00217F1, SARB00638F1, SARB00588F1
7	23	2011071	TESTNOT03	2011071H1 (TESTNOT03), 2011071R6 (TESTNOT03), 2011071T6 (TESTNOT03)
8	24	2186517	PROSNOT26	1286776F1 (BRAINOT11), 2186517H1 (PROSNOT26), 2186517X13C1 (PROSNOT26), 2465845F6 (THYRNOT08), 2615896F6 (GBLANOT01), 3250090H1 (SEMVNOT03), 4820270F6 (PROSTUT17)
9	25	2253585	OVARTUT01	1361776F1 (LUNGNOT12), 1686637T6 (PROSNOT15), 2253585H1 (OVARTUT01), 2822491F6 (ADRETUT06), 2822491T6 (ADRETUT06)
10	26	2447520	THP1NOT03	079381F1 (SYNORAB01), 1616379H1 (BRAITUT12), 2395202H1 (THP1AZT01), 2447520H1 (THP1NOT03), 2682142H1 (SINIUCT01), 2848332H1 (BRSTTUT13), 2856151H1 (CONNNOT02), 3595358H1 (FIBPNOT01), 3702357H1 (PENCNOT07), 3706925H1 (PENCNOT07), 3919689H1 (BRAINOT14)

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 99/27009

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19 and 20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 17, 18
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Claims 17 and 18 were not searched because the claimed compounds were not sufficiently characterised.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

claims 1-20 all partially

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/27009

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-20, all partially

A substantially purified polypeptide comprising the amino acid sequence of SEQ ID No:1, fragments and variants thereof, polynucleotides encoding such, variants, homologues, complements and sequences hybridising thereto, including the polynucleotide of SEQ ID No:17. Expression vectors, host cells, production methods, antibodies and pharmaceutical compositions relating to said polypeptide. Detection methods and methods of treating disorders associated with said polypeptide.

2. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:2 and 18.

3. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:3 and 19.

4. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:4 and 20.

5. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:5 and 21.

6. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:6 and 22.

7. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:7 and 23.

8. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:8 and 24.

9. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:9 and 25.

10. Claims: 1-20, all partially

INTERNATIONAL SEARCH REPORT

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

As invention 1, but relating to SEQ ID Nos:10 and 26.

11. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:11 and 27.

12. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:12 and 28.

13. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:13 and 29.

14. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:14 and 30.

15. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:15 and 31.

16. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:16 and 32.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/27009

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17, 18

Claims 17 and 18 were not searched because the claimed compounds were not sufficiently characterised.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

'ormation on patent family members

International Application No
Pc., US 99/27009

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9800553 A	08-01-1998	US 5958750 A	28-09-1999
		AU 3409897 A	21-01-1998
		US 5854046 A	29-12-1998
		US 6057110 A	02-05-2000
EP 0811687 A	10-12-1997	JP 10057080 A	03-03-1998

Table 1 (cont.)

Protein SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
11	27	2481345	SMCANOT01	2481345F6 (SMCANOT01), 2481345H1 (SMCANOT01), 4099083H2 (BRAITUT26), 4826226H1 (BLADDIT01)
12	28	2484020	SMCANOT01	079652F1 (SYNORAB01), 2308533H1 (NGANNOT01), 2484020H1 (SMCANOT01), 2799586H1 (PENCNOT01), 3571038H1 (HEAPNOT01)
13	29	2862528	SININOT03	403408R1 (TMLR3DT01), 875946R1 (LUNGAST01), 928587R1 (BRAINOT04), 2135155H1 (ENDCNOT01), 2862528H1 (SININOT03)
14	30	3200650	PENCNOT02	1441391F1 (THYRNOT03), 1580635F6 (DUODNOT01), 1726450F6 (PROSNOT14), 2225236T6 (SEMVNOT01), 2995844F6 (OVARTUT07), 3200650F6 (PENCNOT02), 3200650H1 (PENCNOT02), 3319055F6 (PROSBPT03), 3325495H2 (PTHYNOT03), 3358591H1 (PROSTUT16), 3589008H1 (293TF5T01), 4823564H1 (PROSTUT17)
15	31	4107621	BRSTTUT17	1693538F6 (COLNNOT23), 2125414F6 (BRSTNOT07), 2853982H1 (CONNNOT02), 2967492F6 (SCORNOT04), 2967492T6 (SCORNOT04), 4107621H1 (BRSTTUT17), 4648345H1 (PROSTUT20)
16	32	4661133	BRAVTXT03	487982R1 (HNT2AGT01), 1553195X11 (BLADTUT04), 2748561H1 (LUNGTUT11), 4661133H1 (BRAVTXT03), SCAA03254V1, SCAA05351V1, SCAA00669V1

Table 2

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification/ Homologies	Analytical Methods
1	159	S57 S136 S130		Alpha-lactalbumin/lysozyme C motifs: Y24-E34, G37-T61, Y44-A53, D69-D110, S71-N87, N94-V119, C114-C135, G124-C135 Lactalbumin/lysozyme consensus sequence: C96-C114 Lysyzome HMM motif: K22-C147 Lysozyme signature motifs: I23-Y41, H54-H66, T90-K116, N123-G146 Signal peptide: M1-A21	Lysozyme [Paralichthys olivaceus] (GeneSeq W69514) Lysozyme c precursor [Colobus guereza] (GI 1790927) Lysozyme c precursor [Colobus angolensis] (GI 1790967) Lysozyme c precursor [Nasalis larvatis] (GI 1790984)	BLAST BLOCKS MOTIFS PFAM PRINTS SPScan
2	285	T3 T132 T155 T211 S261 S263 S280	N121		NG,NG-dimethylarginine dimethylaminohydrolase [Rattus norvegicus] (GI 1906800)	BLAST MOTIFS
3	331	S290 T94 T156 T216 T303 S308 S322 S52 T123 T225	N168 N198	Protein phosphodiesterases domain: R64-S74, P78-D113 Signal peptide: M1-R26	Similarity to E. coli glycerophosphoryldiester phosphodiesterase [C. elegans] (GI 3877620)	BLAST HMM MOTIFS PRODOM SPScan

Table 2 (cont.)

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification/ Homologies	Analytical Methods
4	153	T31		Signal peptide: M1-S21	Colipase [Myocastor coypus] (GI 599867)	BLAST HMM MOTIFS SPScan
5	571	T27 S119 S131 T158 S187 T188 S285 S324 S366 T379 T390 S398 T486 T53 S106 S139 S304 S477	N105	Carboxylesterase domain: R6-W547 Signal peptide: M1-T27	Carboxylesterase [Mus musculus] (GI 404389)	BLAST BLOCKS HMM MOTIFS PFAM ProfileScan SPScan
6	347	S39 S274 S323	N79 N186 N269 N306 N325	Leucine rich repeat domains: K93-A140, T141-T188, L189-P236, D237-G284 Signal peptide: M1-A35	Phospholipase A2 inhibitor [Agkistrodon blomhoffii siniticus] (GI 3358089)	BLAST BLOCKS MOTIFS PFAM PRINTS SPScan
7	194	S18 T61 T109 T23	N104	C-type lysozyme/alpha-lactalbumin family domain: K66-C191	Lysozyme c precursor [Erythrocebus patas] (GI 1791001)	BLAST BLOCKS MOTIFS Pfam PRINTS ProfileScan

Table 2 (cont.)

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification/ Homologies	Analytical Methods
8	361	T304 S172 S249 T308 T337 S29 S47 T147 S161 Y170	N106	Metallo-beta-lactamase family domain: V97-H267 Signal peptide: M1-A35	Glyoxalase II [Homo sapiens] (GI 1237213)	BLAST BLOCKS MOTIFS PFAM SPScan
9	306	S158 S47 T84		Alpha/beta hydrolase fold: R86-L305 ATP/GTP-binding site motif A (P-loop): G130-T137	Similar to alpha/beta hydrolase fold [C. elegans] (GI 3878848)	BLAST MOTIFS PFAM PRINTS
10	483	S99 T118 T185 S279 S360 S2 S37 T95 T326	N317		Mitochondrial very-long-chain acyl-CoA thioesterase [Rattus norvegicus] (GI 2832739)	BLAST MOTIFS
11	144	T111 T123 S139 Y109	N108	Sulfatases protein domains: P32-F48, P80-L91, G120-D130 Signal Peptide: M1-G22	Bone-related sulphatase-like precursor [Mus musculus] (GeneSeq R51355)	BLAST BLOCKS HMM MOTIFS SPScan
12	180	S28 T99 S158 S39 S178	N7 N153	mutT domain: V34-V73 MutT domain signature: G51-E70	Diphosphoinositol polyphosphate phosphohydrolase [Homo sapiens] (GI 3978224)	BLAST BLOCKS MOTIFS PFAM PRINTS

Table 2 (cont.)

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification/ Homologies	Analytical Methods
13	375	T145 S34 S172 T312 S78 T121 S156 T211 S254 S261 S280 S340 S362	N262	Signal peptide: M1-A29	Acyl-CoA thioester hydrolase [Homo sapiens] (GI 1906670)	BLAST HMM MOTIFS SPScan
14	637	S142 S254 T341 S396 S541 T561 S5 T117 T164 T356 S410 T468		Glycosyl hydrolase family domain: A11-H629 Signal peptide: M1-R28	Beta-galactosidase [Bacillus circulans] (GI 2289790)	BLAST BLOCKS HMM MOTIFS PFAM PRINTS SPScan
15	314	S46 T120 S257 T272 T148 S171 T199 S232 T268	N44 N255	Alpha/beta hydrolase fold: F59-L304	Kraken [Drosophila melanogaster] (GI 2274926)	BLAST MOTIFS PFAM PRINTS
16	448	S95 S105 T186 S232 S263 T269 S276 S388 S406 T417 T119 S145 S284 T439 Y424			Acyl-CoA thioesterase [Mus musculus] (GI 5102774)	BLAST MOTIFS

Table 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	*ESTs:* Probability value= 1.0E-8 or less *Full Length sequences*: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises as least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	*ESTs:* fasta E value=1.06E-6 *Assembled ESTs*: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less *Full Length sequences:* fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S and J.G. Henikoff, Nucl. Acid Res., 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and, if applicable, Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits for PFAM hits, depending on individual protein families

Table 5 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Normalized quality score≥ GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=3.5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. supra; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

What is claimed is:

1. A substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and fragments thereof.

2. A substantially purified variant having at least 90% amino acid sequence identity to the amino acid sequence of claim 1.

3. An isolated and purified polynucleotide encoding the polypeptide of claim 1.

4. An isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide of claim 3.

5. An isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide of claim 3.

6. An isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide of claim 3.

7. A method for detecting a polynucleotide, the method comprising the steps of:

(a) hybridizing the polynucleotide of claim 6 to at least one nucleic acid in a sample, thereby forming a hybridization complex; and

(b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of the polynucleotide in the sample.

8. The method of claim 7 further comprising amplifying the polynucleotide prior to hybridization.

9. An isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:17-32 and fragments thereof.

10. An isolated and purified polynucleotide variant having at least 90% polynucleotide

sequence identity to the polynucleotide of claim 9.

11. An isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide of claim 9.

12. An expression vector comprising at least a fragment of the polynucleotide of claim 3.

13. A host cell comprising the expression vector of claim 12.

14. A method for producing a polypeptide, the method comprising the steps of:

a) culturing the host cell of claim 13 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell culture.

15. A pharmaceutical composition comprising the polypeptide of claim 1 in conjunction with a suitable pharmaceutical carrier.

16. A purified antibody which specifically binds to the polypeptide of claim 1.

17. A purified agonist of the polypeptide of claim 1.

18. A purified antagonist of the polypeptide of claim 1.

19. A method for treating or preventing a disorder associated with decreased expression or activity of HYDRL, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 15.

20. A method for treating or preventing a disorder associated with increased expression or activity of HYDRL, the method comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 18.

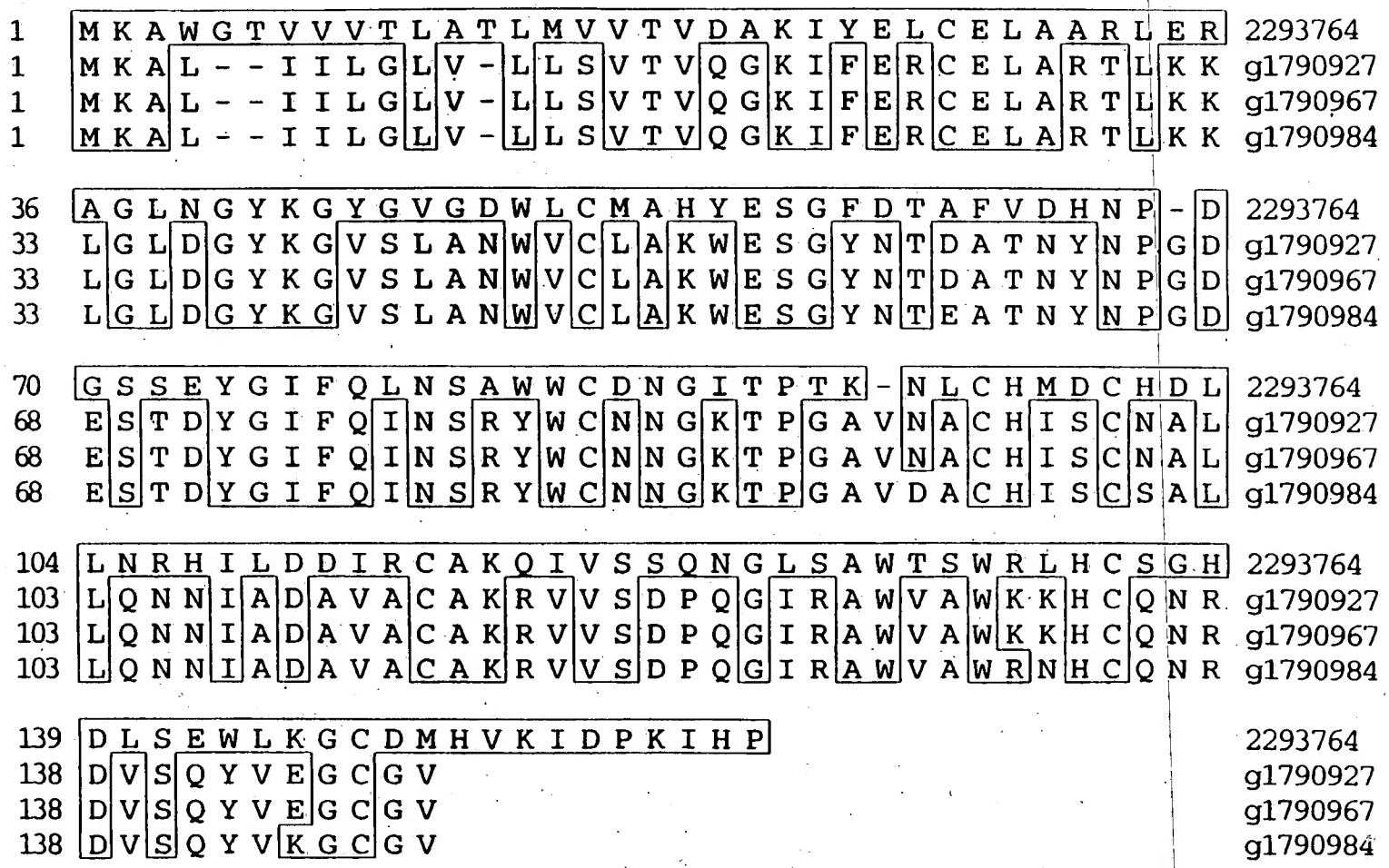
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FIGURE 1

SEQUENCE LISTING

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TANG, Y. Tom
HILLMAN, Jennifer L.
YUE, Henry
LAL, Preeti
BANDMAN, Olga
CORLEY, Neil C.
GUEGLER, Karl J.
BAUGHN, Mariah R.
LU, Dyung Aina M.
AZIMZAI, Yalda
YANG, Junming

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Gly Ile Pro Val Leu Met His Asp Asn Thr Val Asp Arg Thr Thr
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Lys Leu Asn Pro Ala Ala Asn His Arg Leu Arg Asn Asp Phe Pro
140 145 150
Asp Glu Lys Ile Pro Thr Leu Arg Glu Ala Val Ala Glu Cys Leu
155 160 165
Asn His Asn Leu Thr Ile Phe Phe Asp Val Lys Gly His Ala His
170 175 180
Lys Ala Thr Glu Ala Leu Lys Lys Met Tyr Met Glu Phe Pro Gln
185 190 195
Leu Tyr Asn Asn Ser Val Val Cys Ser Phe Leu Pro Glu Val Ile
200 205 210
Tyr Lys Met Arg Gln Thr Asp Arg Asp Val Ile Thr Ala Leu Thr
215 220 225
His Arg Pro Trp Ser Leu Ser His Thr Gly Asp Gly Lys Pro Arg
230 235 240
Tyr Asp Thr Phe Trp Lys His Phe Ile Phe Val Met Met Asp Ile
245 250 255
Leu Leu Asp Trp Ser Met His Asn Ile Leu Trp Tyr Leu Cys Gly
260 265 270
Ile Ser Ala Phe Leu Met Gln Lys Asp Phe Val Ser Pro Ala Tyr
275 280 285
Leu Lys Lys Trp Ser Ala Lys Gly Ile Gln Val Val Gly Trp Thr
290 295 300
Val Asn Thr Phe Asp Glu Lys Ser Tyr Tyr Glu Ser His Leu Gly

```
                305                 310                 315
Ser Ser Tyr Ile Thr Asp Ser Met Val Glu Asp Cys Glu Pro His
                320                 325                 330
Phe
```

<210> 4
<211> 153
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1553276CD1

<400> 4

```
Met Ala Ala Ala Leu Ala Leu Val Ala Gly Val Leu Ser Gly Ala
  1               5                  10                  15
Val Leu Pro Leu Trp Ser Ala Leu Pro Gln Tyr Lys Lys Lys Ile
                20                  25                  30
Thr Asp Arg Cys Phe His His Ser Glu Cys Tyr Ser Gly Cys Cys
                35                  40                  45
Leu Met Asp Leu Asp Ser Gly Gly Ala Phe Cys Ala Pro Arg Ala
                50                  55                  60
Arg Ile Thr Met Ile Cys Leu Pro Gln Trp Leu Glu Leu Phe Lys
                65                  70                  75
Gly Arg Asp Cys Ile Ile Phe Ile Tyr Glu Ala Pro Thr Pro Ser
                80                  85                  90
Leu Val Ser Ala His Asn Gln Gly Ser Tyr Gln His His Leu Pro
                95                 100                 105
Leu Pro Asp Gly Leu Asp Val His Ile Gln Gly Leu Asp Val Phe
               110                 115                 120
Pro Pro Val Pro Tyr Asp Leu Glu Glu Asp Ala Gly Trp Ser Leu
               125                 130                 135
Leu Pro Trp Gly His Arg Pro Trp Leu Pro Pro Thr Cys Ser Lys
               140                 145                 150
Ser Ser Ser
```

<210> 5
<211> 571
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1702211CD1

<400> 5

```
Met Glu Arg Ala Val Arg Val Glu Ser Gly Val Leu Val Gly Val
  1               5                  10                  15
Val Cys Leu Leu Leu Ala Cys Pro Ala Thr Ala Thr Gly Pro Glu
                20                  25                  30
Val Ala Gln Pro Glu Val Asp Thr Thr Leu Gly Arg Val Arg Gly
                35                  40                  45
```

```
Arg Gln Val Gly Val Lys Gly Thr Asp Arg Leu Val Asn Val Phe
                50                  55                  60
Leu Gly Ile Pro Phe Ala Gln Pro Pro Leu Gly Pro Asp Arg Phe
                65                  70                  75
Ser Ala Pro His Pro Ala Gln Pro Trp Glu Gly Val Arg Asp Ala
                80                  85                  90
Ser Thr Ala Pro Pro Met Cys Leu Gln Asp Val Glu Ser Met Asn
                95                 100                 105
Ser Ser Arg Phe Val Leu Asn Gly Lys Gln Gln Ile Phe Ser Val
               110                 115                 120
Ser Glu Asp Cys Leu Val Leu Asn Val Tyr Ser Pro Ala Glu Val
               125                 130                 135
Pro Ala Gly Ser Gly Arg Pro Val Met Val Trp Val His Gly Gly
               140                 145                 150
Ala Leu Ile Thr Gly Ala Ala Thr Ser Tyr Asp Gly Ser Ala Leu
               155                 160                 165
Ala Ala Tyr Gly Asp Val Val Val Val Thr Val Gln Tyr Arg Leu
               170                 175                 180
Gly Val Leu Gly Phe Phe Ser Thr Gly Asp Glu His Ala Pro Gly
               185                 190                 195
Asn Gln Gly Phe Leu Asp Val Val Ala Ala Leu Arg Trp Val Gln
               200                 205                 210
Glu Asn Ile Ala Pro Phe Gly Gly Asp Leu Asn Cys Val Thr Val
               215                 220                 225
Phe Gly Gly Ser Ala Gly Gly Ser Ile Ile Ser Gly Leu Val Leu
               230                 235                 240
Ser Pro Val Ala Ala Gly Leu Phe His Arg Ala Ile Thr Gln Ser
               245                 250                 255
Gly Val Ile Thr Thr Pro Gly Ile Ile Asp Ser His Pro Trp Pro
               260                 265                 270
Leu Ala Gln Lys Ile Ala Asn Thr Leu Ala Cys Ser Ser Ser Ser
               275                 280                 285
Pro Ala Glu Met Val Gln Cys Leu Gln Gln Lys Glu Gly Glu Glu
               290                 295                 300
Leu Val Leu Ser Lys Lys Leu Lys Asn Thr Ile Tyr Pro Leu Thr
               305                 310                 315
Val Asp Gly Thr Val Phe Pro Lys Ser Pro Lys Glu Leu Leu Lys
               320                 325                 330
Glu Lys Pro Phe His Ser Val Pro Phe Leu Met Gly Val Asn Asn
               335                 340                 345
His Glu Phe Ser Trp Leu Ile Pro Arg Gly Trp Gly Leu Leu Asp
               350                 355                 360
Thr Met Glu Gln Met Ser Arg Glu Asp Met Leu Ala Ile Ser Thr
               365                 370                 375
Pro Val Leu Thr Ser Leu Asp Val Pro Pro Glu Met Met Pro Thr
               380                 385                 390
Val Ile Asp Glu Tyr Leu Gly Ser Asn Ser Asp Ala Gln Ala Lys
               395                 400                 405
Cys Gln Ala Phe Gln Glu Phe Met Gly Asp Val Phe Ile Asn Val
               410                 415                 420
Pro Thr Val Ser Phe Ser Arg Tyr Leu Arg Asp Ser Gly Ser Pro
               425                 430                 435
Val Phe Phe Tyr Glu Phe Gln His Arg Pro Ser Ser Phe Ala Lys
               440                 445                 450
Ile Lys Pro Ala Trp Val Lys Ala Asp His Gly Ala Glu Gly Ala
```

```
                455                 460                 465
Phe Val Phe Gly Gly Pro Phe Leu Met Asp Glu Ser Ser Arg Leu
                470                 475                 480
Ala Phe Pro Glu Ala Thr Glu Glu Glu Lys Gln Leu Ser Leu Thr
                485                 490                 495
Met Met Ala Gln Trp Thr His Phe Ala Arg Thr Gly Asp Pro Asn
                500                 505                 510
Ser Lys Ala Leu Pro Pro Trp Pro Gln Phe Asn Gln Ala Glu Gln
                515                 520                 525
Tyr Leu Glu Ile Asn Pro Val Pro Arg Ala Gly Gln Lys Phe Arg
                530                 535                 540
Glu Ala Trp Met Gln Phe Trp Ser Glu Thr Leu Pro Ser Lys Ile
                545                 550                 555
Gln Gln Trp His Gln Lys Gln Lys Asn Arg Lys Ala Gln Glu Asp
                560                 565                 570
Leu
```

<210> 6
<211> 347
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1859618CD1

<400> 6

```
Met Ser Ser Trp Ser Arg Gln Arg Pro Lys Ser Pro Gly Gly Ile
  1               5                  10                  15
Gln Pro His Val Ser Arg Thr Leu Phe Leu Leu Leu Leu Leu Ala
                20                  25                  30
Ala Ser Ala Trp Gly Val Thr Leu Ser Pro Lys Asp Cys Gln Val
                35                  40                  45
Phe Arg Ser Asp His Gly Ser Ser Ile Ser Cys Gln Pro Pro Ala
                50                  55                  60
Glu Ile Pro Gly Tyr Leu Pro Ala Asp Thr Val His Leu Ala Val
                65                  70                  75
Glu Phe Phe Asn Leu Thr His Leu Pro Ala Asn Leu Leu Gln Gly
                80                  85                  90
Ala Ser Lys Leu Gln Glu Leu His Leu Ser Ser Asn Gly Leu Glu
                95                 100                 105
Ser Leu Ser Pro Glu Phe Leu Arg Pro Val Pro Gln Leu Arg Val
               110                 115                 120
Leu Asp Leu Thr Arg Asn Ala Leu Thr Gly Leu Pro Pro Gly Leu
               125                 130                 135
Phe Gln Ala Ser Ala Thr Leu Asp Thr Leu Val Leu Lys Glu Asn
               140                 145                 150
Gln Leu Glu Val Leu Glu Val Ser Trp Leu His Gly Leu Lys Ala
               155                 160                 165
Leu Gly His Leu Asp Leu Ser Gly Asn Arg Leu Arg Lys Leu Pro
               170                 175                 180
Pro Gly Leu Leu Ala Asn Phe Thr Leu Leu Arg Thr Leu Asp Leu
               185                 190                 195
Gly Glu Asn Gln Leu Glu Thr Leu Pro Pro Asp Leu Leu Arg Gly
```

```
                200                 205                 210
Pro Leu Gln Leu Glu Arg Leu His Leu Glu Gly Asn Lys Leu Gln
                215                 220                 225
Val Leu Gly Lys Asp Leu Leu Leu Pro Gln Pro Asp Leu Arg Tyr
                230                 235                 240
Leu Phe Leu Asn Gly Asn Lys Leu Ala Arg Val Ala Ala Gly Ala
                245                 250                 255
Phe Gln Gly Leu Arg Gln Leu Asp Met Leu Asp Leu Ser Asn Asn
                260                 265                 270
Ser Leu Ala Ser Val Pro Glu Gly Leu Trp Ala Ser Leu Gly Gln
                275                 280                 285
Pro Asn Trp Asp Met Arg Asp Gly Phe Asp Ile Ser Gly Asn Pro
                290                 295                 300
Trp Ile Cys Asp Gln Asn Leu Ser Asp Leu Tyr Arg Trp Leu Gln
                305                 310                 315
Ala Gln Lys Asp Lys Met Phe Ser Gln Asn Asp Thr Arg Cys Ala
                320                 325                 330
Gly Pro Glu Ala Val Lys Gly Gln Thr Leu Leu Ala Val Ala Lys
                335                 340                 345
Ser Gln


<210> 7
<211> 194
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2011071CD1

<400> 7
Met Gln Asp Ala Pro Leu Ser Cys Leu Ser Pro Thr Arg Trp Ser
  1               5                  10                  15
Ser Val Ser Ser Ala Asp Ser Thr Glu Lys Ser Ala Ser Gly Ala
                20                  25                  30
Gly Thr Arg Asn Leu Pro Phe Gln Phe Cys Leu Arg Gln Ala Leu
                35                  40                  45
Arg Met Lys Ala Ala Gly Ile Leu Thr Leu Ile Gly Cys Leu Val
                50                  55                  60
Thr Gly Ala Glu Ser Lys Ile Tyr Thr Arg Cys Lys Leu Ala Lys
                65                  70                  75
Ile Phe Ser Arg Ala Gly Leu Asp Asn Tyr Trp Gly Phe Ser Leu
                80                  85                  90
Gly Asn Trp Ile Cys Met Ala Tyr Tyr Glu Ser Gly Tyr Asn Thr
                95                 100                 105
Thr Ala Pro Thr Val Leu Asp Asp Gly Ser Ile Asp Tyr Gly Ile
               110                 115                 120
Phe Gln Ile Asn Thr Phe Ala Trp Cys Arg Arg Gly Lys Leu Lys
               125                 130                 135
Glu Asn Asn His Cys His Val Ala Cys Ser Ala Leu Ile Thr Asp
               140                 145                 150
Asp Leu Thr Asp Ala Ile Ile Cys Ala Arg Lys Ile Val Lys Glu
               155                 160                 165
Thr Gln Gly Met Asn Tyr Trp Gln Gly Trp Lys Lys His Cys Glu
```

```
                170                 175                 180
Gly Arg Asp Leu Ser Glu Trp Lys Lys Gly Cys Glu Val Ser
                185                 190
```

<210> 8
<211> 361
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2186517CD1

<400> 8

```
Met Ala Trp Gln Gly Trp Pro Ala Ala Trp Gln Trp Val Ala Gly
  1               5                  10                  15
Cys Trp Leu Leu Leu Val Leu Val Leu Val Leu Leu Val Ser Pro
                20                  25                  30
Arg Gly Cys Arg Ala Arg Arg Gly Leu Arg Gly Leu Leu Met Ala
                35                  40                  45
His Ser Gln Arg Leu Leu Phe Arg Ile Gly Tyr Ser Leu Tyr Thr
                50                  55                  60
Arg Thr Trp Leu Gly Tyr Leu Phe Tyr Arg Gln Gln Leu Arg Arg
                65                  70                  75
Ala Arg Asn Arg Tyr Pro Lys Gly His Ser Lys Thr Gln Thr Arg
                80                  85                  90
Leu Phe Asn Gly Val Lys Val Leu Pro Ile Pro Val Leu Ser Asp
                95                 100                 105
Asn Tyr Ser Tyr Leu Ile Ile Asp Thr Gln Ala Gln Leu Ala Val
               110                 115                 120
Ala Val Asp Pro Ser Asp Pro Arg Ala Val Gln Ala Ser Ile Glu
               125                 130                 135
Lys Glu Gly Val Thr Leu Val Ala Ile Leu Cys Thr His Lys His
               140                 145                 150
Trp Asp His Ser Gly Gly Asn Arg Asp Leu Ser Arg Arg His Arg
               155                 160                 165
Asp Cys Arg Val Tyr Gly Ser Pro Gln Asp Gly Ile Pro Tyr Leu
               170                 175                 180
Thr His Pro Leu Cys His Gln Asp Val Val Ser Val Gly Arg Leu
               185                 190                 195
Gln Ile Arg Ala Leu Ala Thr Pro Gly His Thr Gln Gly His Leu
               200                 205                 210
Val Tyr Leu Leu Asp Gly Glu Pro Tyr Lys Gly Pro Ser Cys Leu
               215                 220                 225
Phe Ser Gly Asp Leu Leu Phe Leu Ser Gly Cys Gly Arg Thr Phe
               230                 235                 240
Glu Gly Asn Ala Glu Thr Met Leu Ser Ser Leu Asp Thr Val Leu
               245                 250                 255
Gly Leu Gly Asp Asp Thr Leu Leu Trp Pro Gly His Glu Tyr Ala
               260                 265                 270
Glu Glu Asn Leu Gly Phe Ala Gly Val Val Glu Pro Glu Asn Leu
               275                 280                 285
Ala Arg Glu Arg Lys Met Gln Trp Val Gln Arg Gln Arg Leu Glu
               290                 295                 300
```

```
Arg Lys Gly Thr Cys Pro Ser Thr Leu Gly Glu Glu Arg Ser Tyr
                305                 310                 315
Asn Pro Phe Leu Arg Thr His Cys Leu Ala Leu Gln Glu Ala Leu
                320                 325                 330
Gly Pro Gly Pro Gly Pro Thr Gly Asp Asp Asp Tyr Ser Arg Ala
                335                 340                 345
Gln Leu Leu Glu Glu Leu Arg Arg Leu Lys Asp Met His Lys Ser
                350                 355                 360
Lys
```

<210> 9
<211> 306
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2253585CD1

<400> 9

```
Met Leu Arg Trp Thr Arg Ala Trp Arg Leu Pro Arg Glu Gly Leu
  1               5                  10                  15
Gly Pro His Gly Pro Ser Phe Ala Arg Val Pro Val Ala Pro Ser
                20                  25                  30
Ser Ser Ser Gly Gly Arg Gly Gly Ala Glu Pro Arg Pro Leu Pro
                35                  40                  45
Leu Ser Tyr Arg Leu Leu Asp Gly Glu Ala Ala Leu Pro Ala Val
                50                  55                  60
Val Phe Leu His Gly Leu Phe Gly Ser Lys Thr Asn Phe Asn Ser
                65                  70                  75
Ile Ala Lys Ile Leu Ala Gln Gln Thr Gly Arg Arg Val Leu Thr
                80                  85                  90
Val Asp Ala Arg Asn His Gly Asp Ser Pro His Ser Pro Asp Met
                95                 100                 105
Ser Tyr Glu Ile Met Ser Gln Asp Leu Gln Asp Leu Leu Pro Gln
               110                 115                 120
Leu Gly Leu Val Pro Cys Val Val Val Gly His Ser Met Gly Gly
               125                 130                 135
Lys Thr Ala Met Leu Leu Ala Leu Gln Arg Pro Glu Leu Val Glu
               140                 145                 150
Arg Leu Ile Ala Val Asp Ile Ser Pro Val Glu Ser Thr Gly Val
               155                 160                 165
Ser His Phe Ala Thr Tyr Val Ala Ala Met Arg Ala Ile Asn Ile
               170                 175                 180
Ala Asp Glu Leu Pro Arg Ser Arg Ala Arg Lys Leu Ala Asp Glu
               185                 190                 195
Gln Leu Ser Ser Val Ile Gln Asp Met Ala Val Arg Gln His Leu
               200                 205                 210
Leu Thr Asn Leu Val Glu Val Asp Gly Arg Phe Val Trp Arg Val
               215                 220                 225
Asn Leu Asp Ala Leu Thr Gln His Leu Asp Lys Ile Leu Ala Phe
               230                 235                 240
Pro Gln Arg Gln Glu Ser Tyr Leu Gly Pro Thr Leu Phe Leu Leu
               245                 250                 255
```

```
Gly Gly Asn Ser Gln Phe Val His Pro Ser His His Pro Glu Ile
                260                 265                 270
Met Arg Leu Phe Pro Arg Ala Gln Met Gln Thr Val Pro Asn Ala
                275                 280                 285
Gly His Trp Ile His Ala Asp Arg Pro Gln Asp Phe Ile Ala Ala
                290                 295                 300
Ile Arg Gly Phe Leu Val
                305
```

<210> 10
<211> 483
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2447520CD1

<400> 10

```
Met Ser Asn Lys Leu Leu Ser Pro His Pro His Ser Val Val Leu
  1               5                  10                  15
Arg Ser Glu Phe Lys Met Ala Ser Ser Pro Ala Val Leu Arg Ala
                20                  25                  30
Ser Arg Leu Tyr Gln Trp Ser Leu Lys Ser Ser Ala Gln Phe Leu
                35                  40                  45
Gly Ser Pro Gln Leu Arg Gln Val Gly Gln Ile Ile Arg Val Pro
                50                  55                  60
Ala Arg Met Ala Ala Thr Leu Ile Leu Glu Pro Ala Gly Arg Cys
                65                  70                  75
Cys Trp Asp Glu Pro Val Arg Ile Ala Val Arg Gly Leu Ala Pro
                80                  85                  90
Glu Gln Pro Val Thr Leu Arg Ala Ser Leu Arg Asp Glu Lys Gly
                95                 100                 105
Ala Leu Phe Gln Ala His Ala Arg Tyr Arg Ala Asp Thr Leu Gly
               110                 115                 120
Glu Leu Asp Leu Glu Arg Ala Pro Ala Leu Gly Gly Ser Phe Ala
               125                 130                 135
Gly Leu Glu Pro Met Gly Leu Leu Trp Ala Leu Glu Pro Glu Lys
               140                 145                 150
Pro Leu Val Arg Leu Val Lys Arg Asp Val Arg Thr Pro Leu Ala
               155                 160                 165
Val Glu Leu Glu Val Leu Asp Gly His Asp Pro Asp Pro Gly Arg
               170                 175                 180
Leu Leu Cys Gln Thr Arg His Glu Arg Tyr Phe Leu Pro Pro Gly
               185                 190                 195
Val Arg Arg Glu Pro Val Arg Val Gly Arg Val Arg Gly Thr Leu
               200                 205                 210
Phe Leu Pro Pro Glu Pro Gly Pro Phe Pro Gly Ile Val Asp Met
               215                 220                 225
Phe Gly Thr Gly Gly Gly Leu Leu Glu Tyr Arg Ala Ser Leu Leu
               230                 235                 240
Ala Gly Lys Gly Phe Ala Val Met Ala Leu Ala Tyr Tyr Asn Tyr
               245                 250                 255
Glu Asp Leu Pro Lys Thr Met Glu Thr Leu His Leu Glu Tyr Phe
```

```
                260                 265                 270
Glu Glu Ala Met Asn Tyr Leu Leu Ser His Pro Glu Val Lys Gly
                275                 280                 285
Pro Gly Val Gly Leu Leu Gly Ile Ser Lys Gly Gly Glu Leu Cys
                290                 295                 300
Leu Ser Met Ala Ser Phe Leu Lys Gly Ile Thr Ala Ala Val Val
                305                 310                 315
Ile Asn Gly Ser Val Ala Asn Val Gly Gly Thr Leu Arg Tyr Lys
                320                 325                 330
Gly Glu Thr Leu Pro Pro Val Gly Val Asn Arg Asn Arg Ile Lys
                335                 340                 345
Val Thr Lys Asp Gly Tyr Ala Asp Ile Val Asp Val Leu Asn Ser
                350                 355                 360
Pro Leu Glu Gly Pro Asp Gln Lys Ser Phe Ile Pro Val Glu Arg
                365                 370                 375
Ala Glu Ser Thr Phe Leu Phe Leu Val Gly Gln Asp Asp His Asn
                380                 385                 390
Trp Lys Ser Glu Phe Tyr Ala Asn Glu Ala Cys Lys Arg Leu Gln
                395                 400                 405
Ala His Gly Arg Arg Lys Pro Gln Ile Ile Cys Tyr Pro Glu Thr
                410                 415                 420
Gly His Tyr Ile Glu Pro Pro Tyr Phe Pro Leu Cys Arg Ala Ser
                425                 430                 435
Leu His Ala Leu Val Gly Ser Pro Ile Ile Trp Gly Gly Glu Pro
                440                 445                 450
Arg Ala His Ala Met Ala Gln Val Asp Ala Trp Lys Gln Leu Gln
                455                 460                 465
Thr Phe Phe His Lys His Leu Gly Gly His Glu Gly Thr Ile Pro
                470                 475                 480
Ser Lys Val
```

<210> 11
<211> 144
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2481345CD1

<400> 11

```
Met Leu Leu Leu Trp Val Ser Val Val Ala Ala Leu Ala Leu Ala
  1                 5                  10                  15
Val Leu Ala Pro Gly Ala Gly Glu Gln Arg Arg Arg Ala Ala Lys
                 20                  25                  30
Ala Pro Asn Val Val Leu Val Val Ser Asp Ser Phe Asp Gly Arg
                 35                  40                  45
Leu Thr Phe His Pro Gly Ser Gln Val Val Lys Leu Pro Phe Ile
                 50                  55                  60
Asn Phe Met Lys Thr Arg Gly Thr Ser Phe Leu Asn Ala Tyr Thr
                 65                  70                  75
Asn Ser Pro Ile Cys Cys Pro Ser Arg Ala Ala Met Trp Ser Gly
                 80                  85                  90
Leu Phe Thr His Leu Thr Glu Ser Trp Asn Asn Phe Lys Gly Leu
```

```
                 95                  100                 105
Asp Pro Asn Tyr Thr Thr Trp Met Asp Val Met Glu Arg His Gly
                110                 115                 120
Tyr Arg Thr Gln Lys Phe Gly Lys Leu Asp Tyr Thr Ser Gly His
                125                 130                 135
His Ser Ile Ser Asn Arg Val Glu Ala
                140
```

<210> 12
<211> 180
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2484020CD1

<400> 12

```
Met Met Lys Phe Lys Pro Asn Gln Thr Arg Thr Tyr Asp Arg Glu
  1               5                  10                  15
Gly Phe Lys Lys Arg Ala Ala Cys Leu Cys Phe Arg Ser Glu Gln
                20                  25                  30
Glu Asp Glu Val Leu Leu Val Ser Ser Ser Arg Tyr Pro Asp Gln
                35                  40                  45
Trp Ile Val Pro Gly Gly Gly Met Glu Pro Glu Glu Glu Pro Gly
                50                  55                  60
Gly Ala Ala Val Arg Glu Val Tyr Glu Glu Ala Gly Val Lys Gly
                65                  70                  75
Lys Leu Gly Arg Leu Leu Gly Ile Phe Glu Asn Gln Asp Arg Lys
                80                  85                  90
His Arg Thr Tyr Val Tyr Val Leu Thr Val Thr Glu Ile Leu Glu
                95                 100                 105
Asp Trp Glu Asp Ser Val Asn Ile Gly Arg Lys Arg Glu Trp Phe
               110                 115                 120
Lys Val Glu Asp Ala Ile Lys Val Leu Gln Cys His Lys Pro Val
               125                 130                 135
His Ala Glu Tyr Leu Glu Lys Leu Lys Leu Gly Cys Ser Pro Ala
               140                 145                 150
Asn Gly Asn Ser Thr Val Pro Ser Leu Pro Asp Asn Asn Ala Leu
               155                 160                 165
Phe Val Thr Ala Ala Gln Thr Ser Gly Leu Pro Ser Ser Val Arg
               170                 175                 180
```

<210> 13
<211> 375
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2862528CD1

<400> 13
Met Ala Arg Pro Gly Leu Ile His Ser Ala Pro Gly Leu Pro Asp
1 5 10 15
Thr Cys Ala Leu Leu Gln Pro Pro Ala Ala Ser Ala Ala Ala Ala
20 25 30
Pro Ser Met Ser Gly Pro Asp Val Glu Thr Pro Ser Ala Ile Gln
35 40 45
Ile Cys Arg Ile Met Arg Pro Asp Asp Ala Asn Val Ala Gly Asn
50 55 60
Val His Gly Gly Thr Ile Leu Lys Met Ile Glu Glu Ala Gly Ala
65 70 75
Ile Ile Ser Thr Arg His Cys Asn Ser Gln Asn Gly Glu Arg Cys
80 85 90
Val Ala Ala Leu Ala Arg Val Glu Arg Thr Asp Phe Leu Ser Pro
95 100 105
Met Cys Ile Gly Glu Val Ala His Val Ser Ala Glu Ile Thr Tyr
110 115 120
Thr Ser Lys His Ser Val Glu Val Gln Val Asn Val Met Ser Glu
125 130 135
Asn Ile Leu Thr Gly Ala Lys Lys Leu Thr Asn Lys Ala Thr Leu
140 145 150
Trp Tyr Val Pro Leu Ser Leu Lys Asn Val Asp Lys Val Leu Glu
155 160 165
Val Pro Pro Val Val Tyr Ser Arg Gln Glu Gln Glu Glu Glu Gly
170 175 180
Arg Lys Arg Tyr Glu Ala Gln Lys Leu Glu Arg Met Glu Thr Lys
185 190 195
Trp Arg Asn Gly Asp Ile Val Gln Pro Val Leu Asn Pro Gly Val
200 205 210
Thr Met Lys Leu Met Asp Glu Val Ala Gly Ile Val Ala Ala Arg
215 220 225
His Cys Lys Thr Asn Ile Val Thr Ala Ser Val Asp Ala Ile Asn
230 235 240
Phe His Asp Lys Ile Arg Lys Gly Cys Val Ile Thr Ile Ser Gly
245 250 255
Arg Met Thr Phe Thr Ser Asn Lys Ser Met Glu Ile Glu Val Leu
260 265 270
Val Asp Ala Asp Pro Val Val Asp Ser Ser Gln Lys Arg Tyr Arg
275 280 285
Ala Ala Ser Ala Phe Phe Thr Tyr Val Ser Leu Ser Gln Glu Gly
290 295 300
Arg Ser Leu Pro Val Pro Gln Leu Val Pro Glu Thr Glu Asp Glu
305 310 315
Lys Lys Arg Phe Glu Glu Gly Lys Gly Arg Tyr Leu Gln Met Lys
320 325 330
Ala Asn Asp Arg Ala Thr Arg Ser Leu Ser Pro Arg Leu Pro Pro
335 340 345
Pro Ala Thr Gly Ala Ser Ser Ser His Gly Asn Gly Pro Ser Val
350 355 360
Gln Ser Leu Arg Ser Ser Pro Leu Gly Gln Lys Pro Asn Ser His
365 370 375

<210> 14
<211> 637

<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 3200650CD1

<400> 14

```
Met Thr Thr Trp Ser Leu Arg Arg Arg Pro Ala Arg Thr Leu Gly
  1               5                  10                  15
Leu Leu Leu Leu Val Val Leu Gly Phe Leu Val Leu Arg Arg Leu
             20                  25                  30
Asp Trp Ser Thr Leu Val Pro Leu Arg Leu Arg His Arg Gln Leu
             35                  40                  45
Gly Leu Gln Ala Lys Gly Trp Asn Phe Met Leu Glu Asp Ser Thr
             50                  55                  60
Phe Trp Ile Phe Gly Gly Ser Ile His Tyr Phe Arg Val Pro Arg
             65                  70                  75
Glu Tyr Trp Arg Asp Arg Leu Leu Lys Met Lys Ala Cys Gly Leu
             80                  85                  90
Asn Thr Leu Thr Thr Tyr Val Pro Trp Asn Leu His Glu Pro Glu
             95                 100                 105
Arg Gly Lys Phe Asp Phe Leu Trp Glu Thr Trp Thr Leu Lys Ala
            110                 115                 120
Phe Val Leu Met Ala Ala Glu Ile Gly Leu Trp Val Ile Leu Arg
            125                 130                 135
Pro Gly Pro Tyr Ile Cys Ser Glu Met Asp Leu Gly Gly Leu Pro
            140                 145                 150
Ser Trp Leu Leu Gln Asp Pro Gly Met Arg Leu Arg Thr Thr Tyr
            155                 160                 165
Lys Gly Phe Thr Glu Ala Val Asp Leu Tyr Phe Asp His Leu Met
            170                 175                 180
Ser Arg Val Val Pro Leu Gln Tyr Lys Arg Gly Gly Pro Ile Ile
            185                 190                 195
Ala Val Gln Val Glu Asn Glu Tyr Gly Ser Tyr Asn Lys Asp Pro
            200                 205                 210
Ala Tyr Met Pro Tyr Val Lys Lys Ala Leu Glu Asp Arg Gly Ile
            215                 220                 225
Val Glu Leu Leu Leu Thr Ser Asp Asn Lys Asp Gly Leu Ser Lys
            230                 235                 240
Gly Ile Val Gln Gly Val Leu Ala Thr Ile Asn Leu Gln Ser Thr
            245                 250                 255
His Glu Leu Gln Leu Leu Thr Thr Phe Leu Phe Asn Val Gln Gly
            260                 265                 270
Thr Gln Pro Lys Met Val Met Glu Tyr Trp Thr Gly Trp Phe Asp
            275                 280                 285
Ser Trp Gly Gly Pro His Asn Ile Leu Asp Ser Ser Glu Val Leu
            290                 295                 300
Lys Thr Val Ser Ala Ile Val Asp Ala Gly Ser Ser Ile Asn Leu
            305                 310                 315
Tyr Met Phe His Gly Gly Thr Asn Phe Gly Phe Met Asn Gly Ala
            320                 325                 330
Met His Phe His Asp Tyr Lys Ser Asp Val Thr Ser Tyr Asp Tyr
            335                 340                 345
Asp Ala Val Leu Thr Glu Ala Gly Asp Tyr Thr Ala Lys Tyr Met
```

```
                350                 355                 360
Lys Leu Arg Asp Phe Phe Gly Ser Ile Ser Gly Ile Pro Leu Pro
                365                 370                 375
Pro Pro Pro Asp Leu Leu Pro Lys Met Pro Tyr Glu Pro Leu Thr
                380                 385                 390
Pro Val Leu Tyr Leu Ser Leu Trp Asp Ala Leu Lys Tyr Leu Gly
                395                 400                 405
Glu Pro Ile Lys Ser Glu Lys Pro Ile Asn Met Glu Asn Leu Pro
                410                 415                 420
Val Asn Gly Gly Asn Gly Gln Ser Phe Gly Tyr Ile Leu Tyr Glu
                425                 430                 435
Thr Ser Ile Thr Ser Ser Gly Ile Leu Ser Gly His Val His Asp
                440                 445                 450
Arg Gly Gln Val Phe Val Asn Thr Val Ser Ile Gly Phe Leu Asp
                455                 460                 465
Tyr Lys Thr Thr Lys Ile Ala Val Pro Leu Ile Gln Gly Tyr Thr
                470                 475                 480
Val Leu Arg Ile Leu Val Glu Asn Arg Gly Arg Val Asn Tyr Gly
                485                 490                 495
Glu Asn Ile Asp Asp Gln Arg Lys Gly Leu Ile Gly Asn Leu Tyr
                500                 505                 510
Leu Asn Asp Ser Pro Leu Lys Asn Phe Arg Ile Tyr Ser Leu Asp
                515                 520                 525
Met Lys Lys Ser Phe Phe Gln Arg Phe Gly Leu Asp Lys Trp Ser
                530                 535                 540
Ser Leu Pro Glu Thr Pro Thr Leu Pro Ala Phe Phe Leu Gly Ser
                545                 550                 555
Leu Ser Ile Ser Ser Thr Pro Cys Asp Thr Phe Leu Lys Leu Glu
                560                 565                 570
Gly Trp Glu Lys Gly Val Val Phe Ile Asn Gly Gln Asn Leu Gly
                575                 580                 585
Arg Tyr Trp Asn Ile Gly Pro Gln Lys Thr Leu Tyr Leu Pro Gly
                590                 595                 600
Pro Trp Leu Ser Ser Gly Ile Asn Gln Val Ile Val Phe Glu Glu
                605                 610                 615
Thr Met Ala Gly Pro Ala Leu Gln Phe Thr Glu Thr Pro His Leu
                620                 625                 630
Gly Arg Asn Gln Tyr Ile Lys
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<210> 15
<211> 314
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 4107621CD1

<400> 15

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Ala Val Pro Trp Gly His Ile Ala Ala Lys Ala Trp Gly Ser Leu
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Gln Gly Pro Pro Val Leu Cys Leu His Gly Trp Leu Asp Asn Ala
                35                  40                  45
Ser Ser Phe Asp Arg Leu Ile Pro Leu Leu Pro Gln Asp Phe Tyr
                50                  55                  60
Tyr Val Ala Met Asp Phe Gly Gly His Gly Leu Ser Ser His Tyr
                65                  70                  75
Ser Pro Gly Val Pro Tyr Tyr Leu Gln Thr Phe Val Ser Glu Ile
                80                  85                  90
Arg Arg Val Val Ala Ala Leu Lys Trp Asn Arg Phe Ser Ile Leu
                95                  100                 105
Gly His Ser Phe Gly Gly Val Val Gly Gly Met Phe Phe Cys Thr
                110                 115                 120
Phe Pro Glu Met Val Asp Lys Leu Ile Leu Leu Asp Thr Pro Leu
                125                 130                 135
Phe Leu Leu Glu Ser Asp Glu Met Glu Asn Leu Leu Thr Tyr Lys
                140                 145                 150
Arg Arg Ala Ile Glu His Val Leu Gln Val Glu Ala Ser Gln Glu
                155                 160                 165
Pro Ser His Val Phe Ser Leu Lys Gln Leu Leu Gln Arg Leu Leu
                170                 175                 180
Lys Ser Asn Ser His Leu Ser Glu Glu Cys Gly Glu Leu Leu Leu
                185                 190                 195
Gln Arg Gly Thr Thr Lys Val Ala Thr Gly Leu Val Leu Asn Arg
                200                 205                 210
Asp Gln Arg Leu Ala Trp Ala Glu Asn Ser Ile Asp Phe Ile Ser
                215                 220                 225
Arg Glu Leu Cys Ala His Ser Ile Arg Lys Leu Gln Ala His Val
                230                 235                 240
Leu Leu Ile Lys Ala Val His Gly Tyr Phe Asp Ser Arg Gln Asn
                245                 250                 255
Tyr Ser Glu Lys Glu Ser Leu Ser Phe Met Ile Asp Thr Met Lys
                260                 265                 270
Ser Thr Leu Lys Glu Gln Phe Gln Phe Val Glu Val Pro Gly Asn
                275                 280                 285
His Cys Val His Met Ser Glu Pro Gln His Val Ala Ser Ile Ile
                290                 295                 300
Ser Ser Phe Leu Gln Cys Thr His Met Leu Pro Ala Gln Leu
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<210> 16
<211> 448
<212> PRT
<213> Homo sapiens

<220>
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<223> Incyte ID No: 4661133CD1

<400> 16

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Lys Gln Gly Ile Phe His Ile His Glu Ala Cys Ser Ser Ile His
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                35                  40                  45
Val Asn His Val Arg Asp Lys Leu Arg Glu Ile Val Gly Ala Ser
                50                  55                  60
Thr Asn Trp Arg Asp His Val Lys Ala Met Glu Glu Arg Lys Leu
                65                  70                  75
Leu His Ser Phe Leu Ala Lys Ser Gln Asp Gly Leu Pro Pro Arg
                80                  85                  90
Arg Met Lys Asp Ser Tyr Ile Glu Val Leu Leu Pro Leu Gly Ser
                95                  100                 105
Glu Pro Glu Leu Arg Glu Lys Tyr Leu Thr Val Gln Asn Thr Val
                110                 115                 120
Arg Phe Gly Arg Ile Leu Glu Asp Leu Asp Ser Leu Gly Val Leu
                125                 130                 135
Ile Cys Tyr Met His Asn Lys Ile His Ser Ala Lys Met Ser Pro
                140                 145                 150
Leu Ser Ile Val Thr Ala Leu Val Asp Lys Ile Asp Met Cys Lys
                155                 160                 165
Lys Ser Leu Ser Pro Glu Gln Asp Ile Lys Phe Ser Gly His Val
                170                 175                 180
Ser Trp Val Gly Lys Thr Ser Met Glu Val Lys Met Gln Met Phe
                185                 190                 195
Gln Leu His Gly Asp Glu Phe Cys Pro Val Leu Asp Ala Thr Phe
                200                 205                 210
Val Met Val Ala Arg Asp Ser Glu Asn Lys Gly Pro Ala Phe Val
                215                 220                 225
Asn Pro Leu Ile Pro Glu Ser Pro Glu Glu Glu Glu Leu Phe Arg
                230                 235                 240
Gln Gly Glu Leu Asn Lys Gly Arg Arg Ile Ala Phe Ser Ser Thr
                245                 250                 255
Ser Leu Leu Lys Met Ala Pro Ser Ala Glu Glu Arg Thr Thr Ile
                260                 265                 270
His Glu Met Phe Leu Ser Thr Leu Asp Pro Lys Thr Ile Ser Phe
                275                 280                 285
Arg Ser Arg Val Leu Pro Ser Asn Ala Val Trp Met Glu Asn Ser
                290                 295                 300
Lys Leu Lys Ser Leu Glu Ile Cys His Pro Gln Glu Arg Asn Ile
                305                 310                 315
Phe Asn Arg Ile Phe Gly Gly Phe Leu Met Arg Lys Ala Tyr Glu
                320                 325                 330
Leu Ala Trp Ala Thr Ala Cys Ser Phe Gly Gly Ser Arg Pro Phe
                335                 340                 345
Val Val Ala Val Asp Asp Ile Met Phe Gln Lys Pro Val Glu Val
                350                 355                 360
Gly Ser Leu Leu Phe Leu Ser Ser Gln Val Cys Phe Thr Gln Asn
                365                 370                 375
Asn Tyr Ile Gln Val Arg Val His Ser Glu Val Ala Ser Leu Gln
                380                 385                 390
Glu Lys Gln His Thr Thr Thr Asn Val Phe His Phe Thr Phe Met
                395                 400                 405
Ser Glu Lys Glu Val Pro Leu Val Phe Pro Lys Thr Tyr Gly Glu
                410                 415                 420
Ser Met Leu Tyr Leu Asp Gly Gln Arg His Phe Asn Ser Met Ser
                425                 430                 435
Gly Pro Ala Thr Leu Arg Lys Asp Tyr Leu Val Glu Pro
                440                 445
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<210> 17
<211> 723
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2293764CB1

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agggtgagtc tggagcaagt cacctgggag ggcttacagg tgccataatg aaggcctggg 180
gcactgtggt agtgaccttg gccacgctga tggttgtcac tgtggatgcc aagatctatg 240
aactctgcga gctggcggca agactggaga gagcagggct gaacggctac aagggctacg 300
gcgttggaga ctggctgtgc atggctcatt atgagagtgg ctttgacacc gccttcgtgg 360
accacaatcc tgatggcagc agtgaatatg gcattttcca actgaattct gcctggtggt 420
gtgacaatgg cattacaccc accaagaacc tctgccacat ggattgtcat gacctgctca 480
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<210> 18
<211> 1228
<212> DNA
<213> Homo sapiens

<220>
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<223> Incyte ID No: 949738CB1

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agggggagtc tgtgtggatg ggatggggac gccgggggag gggctgggcc gctgctccca 300
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gcaacgactg gggctacagc tgctagaact gccacctgag gagtcattgc cgctgggacc 480
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<210> 19
<211> 2155
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1297034CB1

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<210> 20
<211> 491
<212> DNA

<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 155276CB1

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gttcccgccg gtgccatatg atttagagga agatgcaggc tggtcactgc tcccttgggg 420
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<210> 21
<211> 2101
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1702211CB1

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<210> 22
<211> 1834
<212> DNA
<213> Homo sapiens

<220>
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<223> Incyte ID No: 1859618CB1

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<210> 23

<211> 753
<212> DNA
<213> Homo sapiens

<220>
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<223> Incyte ID No: 2011071CB1

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<210> 24
<211> 1395
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2186517CB1

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Thr Asn Tyr Asn Pro Gly Asp Glu Ser Thr Asp Tyr Gly Ile Phe
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        35                  40                  45
Trp Val Cys Leu Ala Lys Trp Glu Ser Gly Tyr Asn Thr Glu Ala
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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60/135,519	21 May 1999 (21.05.99)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

US	60/172,256 (CIP)
Filed on	12 November 1998 (12.11.98)
US	60/135,519 (CIP)
Filed on	21 May 1999 (21.05.99)

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(72) Inventors; and

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

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(54) Title: HUMAN HYDROLASE PROTEINS

(57) Abstract

The invention provides human hydrolase proteins (HYDRL) and polynucleotides which identify and encode HYDRL. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HYDRL.

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INTERNATIONAL SEARCH REPORT

International Application No
I../US 99/27009

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/55 C12N9/14 C12Q1/68 C12N1/21 A61K38/46
C07K16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] AC Z98304, 11 August 1997 (1997-08-11) GRAFHAM D: "Human DNA sequence from clone 54B20 on chromosome Xp11.1-11.3" XP002133630 99.6% identity in 234 nt overlap with SEQ ID No:17 (313-80:109182-109415) ---	3-6,9
X	DATABASE EMBL [Online] AC X60237, 2 December 1991 (1991-12-02) SWANSON KW ET AL: "C. aethiops mRNA for lysozyme" XP002133631 59.3% identity in 428 nt overlap --- -/--	1-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 March 2000	05.07.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lejeune, R

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
P../US 99/27009

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 00553 A (INCYTE PHARMA INC ;AU YOUNG JANICE (US); HAWKINS PHILLIP R (US); H) 8 January 1998 (1998-01-08) abstract	
A	EP 0 811 687 A (HAYASHIBARA BIOCHEM LAB) 10 December 1997 (1997-12-10) abstract	

1

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 99/27009

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19 and 20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 17, 18
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Claims 17 and 18 were not searched because the claimed compounds were not sufficiently characterised.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

claims 1-20 all partially

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/27009

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-20, all partially

A substantially purified polypeptide comprising the amino acid sequence of SEQ ID No:1, fragments and variants thereof, polynucleotides encoding such, variants, homologues, complements and sequences hybridising thereto, including the polynucleotide of SEQ ID No:17. Expression vectors, host cells, production methods, antibodies and pharmaceutical compositions relating to said polypeptide. Detection methods and methods of treating disorders associated with said polypeptide.

2. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:2 and 18.

3. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:3 and 19.

4. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:4 and 20.

5. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:5 and 21.

6. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:6 and 22.

7. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:7 and 23.

8. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:8 and 24.

9. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:9 and 25.

10. Claims: 1-20, all partially

INTERNATIONAL SEARCH REPORT

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

As invention 1, but relating to SEQ ID Nos:10 and 26.

11. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:11 and 27.

12. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:12 and 28.

13. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:13 and 29.

14. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:14 and 30.

15. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:15 and 31.

16. Claims: 1-20, all partially

As invention 1, but relating to SEQ ID Nos:16 and 32.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/27009

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17, 18

Claims 17 and 18 were not searched because the claimed compounds were not sufficiently characterised.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

'ormation on patent family members

International Application No
P.., US 99/27009

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9800553 A	08-01-1998	US 5958750 A	28-09-1999
		AU 3409897 A	21-01-1998
		US 5854046 A	29-12-1998
		US 6057110 A	02-05-2000
EP 0811687 A	10-12-1997	JP 10057080 A	03-03-1998